

We claim:

- 1 1. A method for increasing the production of clavulanic acid in a host comprising the
2 step of:
3 increasing the level of N²-(2-carboxyethyl)arginine synthase in said host, wherein
4 said N²-(2-carboxyethyl)arginine synthase catalyzes the condensation of L-arginine and D-
5 glyceraldehyde-3-phosphate, resulting in increased production of clavulanic acid.
- 1 2. The method of claim 1 wherein said step of increasing is performed by gene dosing.
- 1 3. The method of Claim 2 wherein said increasing step is performed by providing said
2 host with DNA encoding said N²-(2-carboxyethyl)arginine synthase.
- 1 4. The method of claim 3 wherein said DNA is in a plasmid.
- 1 5. The method of claim 4 wherein said plasmid is a replicating plasmid.
- 1 6. The method of claim 5 wherein said replicating plasmid is pKC1139/pro-orf2-ter.
- 1 7. The method of claim 3 further comprising integrating said DNA into the
2 chromosome of said host.
- 1 8. The method of claim 7 wherein said DNA is stably integrated via an integrative
2 vector selected from the group consisting of pSET152/pro-orf2, pSET152/ermE(XbaI)-orf2
3 and pSET152/ermE(HindIII)-orf2.

1 9. The method of claim 3 wherein expression of said DNA is under the control of a
2 constitutive promoter.

1 10. The method of claim 3 wherein said constitutive promoter is *ermE**.

1 11. The method of claim 1 wherein said increasing step is performed by adjusting
2 fermentation conditions and/or providing additives which effect the optimization of
3 N²-(2-carboxyethyl)arginine synthase activity, wherein said optimization results in an
4 increase in the production of clavulanic acid

1 12. The method of claim 1 wherein said host is *Streptomyces clavuligerus*.

1 13. A method for increasing the production of clavulanic acid in a host comprising the
2 step of:

3 increasing the availability of precursors for reaction by N²-(2-carboxyethyl)arginine
4 synthase, wherein said step of increasing results in an increase in the production of
5 clavulanic acid.

1 14. The method of claim 13 wherein said precursors are L-arginine and D-
2 glyceraldehyde-3-phosphate.

1 15. The method of claim 13 wherein said host is *Streptomyces clavuligerus*.

1 16. The method of claim 13 wherein said increasing step is achieved by adjusting
2 fermentation conditions and/or providing additives which optimize
3 N²-(2-carboxyethyl)arginine synthase activity.

1 17. A method for increasing the production of N²-(2-carboxyethyl)arginine in a host
2 cell, comprising,
3 enhancing a rate of condensation of L-arginine and D-glyceraldehyde-3-phosphate in
4 said host cell, wherein said step of enhancing results in an increase in the production of
5 N²-(2-carboxyethyl)arginine in said host cell.

1 18. The method of claim 17 wherein said condensation of L-arginine and D-
2 glyceraldehyde-3-phosphate is catalyzed by the enzyme N²-(2-carboxyethyl)arginine
3 synthase.

1 19. The method of claim 17 wherein said step of enhancing is carried out by increasing
2 the copy number of a gene encoding N²-(2-carboxyethyl)arginine synthase.

1 20. The method of claim 17 wherein said step of enhancing is carried out by adjusting
2 fermentation conditions and/or providing additives which optimize
3 N²-(2-carboxyethyl) arginine synthase activity.

1 21. A method for preparing an composition having N²-(2-carboxyethyl)arginine
2 synthase activity, comprising the steps of
3 growing a culture of a host cell capable of synthesizing N²-(2-carboxyethyl)arginine
4 synthase,
5 harvesting and sonicating said culture,
6 removing cellular debris to produce a cellular supernatant,
7 fractionating said supernatant with ammonium sulfate to form a precipitated protein
8 pellet,
9 resuspending said precipitated protein pellet to form a protein solution, and,
10 chromatographing said protein solution by affinity chromatography to isolate a

11 thiaminepyrophosphate-dependent enzyme having N²-(2-carboxyethyl)arginine synthase
12 activity.

1 22. The method of claim 21 wherein said affinity chromatography is carried out with
2 an L-arginine agarose affinity column.

1 23. The method of claim 21 wherein said host is *Streptomyces clavuligerus*.

1 24. The method of claim 21 wherein said step of fractionating is carried out with 30%
2 ammonium sulfate.

1 25. An assay for identifying substrates of the enzyme N²-(2-carboxyethyl)arginine
2 synthase, comprising the steps of

3 incubating a putative substrate with the enzyme N²-(2-carboxyethyl)arginine
4 synthase, thiaminepyrophosphate, and one known substrate of N²-(2-carboxyethyl)arginine
5 synthase, and

6 detecting the presence or absence of a condensation product of the putative substrate
7 and the known substrate, wherein the presence of a condensation product is a positive result.

1 26. The assay of claim 25 wherein said known substrate is L-arginine.

1 27. The assay of claim 25 wherein said known substrate is D-glyceraldehyde-3-
2 phosphate.

1 28. A host cell stably transformed with *orf2*.

1 29. The host cell of claim 28, wherein said host cell is *Streptomyces clavuligerus*.

30. A condensation product of two substrates condensed by
N²-(2-carboxyethyl)arginine synthase.